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Crystal Structure of the Tetramerization Domain of Acetylcholinesterase at 2.3 Å Resolution

H. Dvir and M. Harel (Weizmann Institute of Science, Israel), W.Q. Liu, C. Garbay (UFR des Sciences Pharmaceutiques et Biologiques, France), J.L. Sussman (Weizmann Institute of Science, Israel), S. Bon and J. Massoulie (Ecole Normale Supèrieure, France), and I. Silman (Weizmann Institute of Science, Israel)

Beamline(s): X4A

Introduction: Hetero-tetramerization of acetylcholinesterase of type T ($AChE_T$) is achieved through interaction of a 40-residue C-terminal domain, the 'tryptophan amphiphilic tetramerization' (WAT) domain, with a 17-residue 'proline-rich attachment domain' (PRAD), at the N-terminus of the ColQ collagen tail in vertebrates, with 4:1 WAT:PRAD stoichiometry (1).

Methods and Materials: The WAT and PRAD peptides were produced by chemical synthesis, with Met21 of WAT being replaced by selenomethionine (SeMet), to facilitate a MAD diffraction experiment. The synthetic WAT and PRAD were mixed at a 4:1 ratio, and co-crystallized. The crystals were cryo-protected by 35 % glycerol and flash cooled to 100 K. The frozen crystals diffracted to 2.3 A resolution, and MAD data were collected. The crystal is in space group P21 with two complexes in the asymmetric unit, resulting in eight independent SeMet residues. The structure was solved by MAD with the program SOLVE, which produced a traceable electron density map. Refinement of the structure in REFMAC and CNS yielded an R-factor of 24.6% and Rf of 29.3 with the 2 PRAD peptides in the asymmetric unit seen in full and the 8 WAT chains, displaying disordered C-termini.

Results: The WAT chains assume an α-helical conformation, and are all parallel. The PRAD has a polyproline II conformation and threads it's way anti-parallel to the WAT chains. Most of the 3 highly conserved Trp residues in each WAT chain are stacked against the 8 Pro residues or 3 Phe residues of the single PRAD (Figure 1). An AChE tetramer structure can be modeled based on the structure of the WAT/PRAD complex.

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References: 1. S. Simon, E. Krejci and J. Massoulie, "A four-to-one association between peptide motifs: four C-terminal domains from cholinesterase assemble with one proline-rich attachment domain (PRAD) in the secretory pathway". The EMBO Journal 17, 21, 6178-6187, 1998.

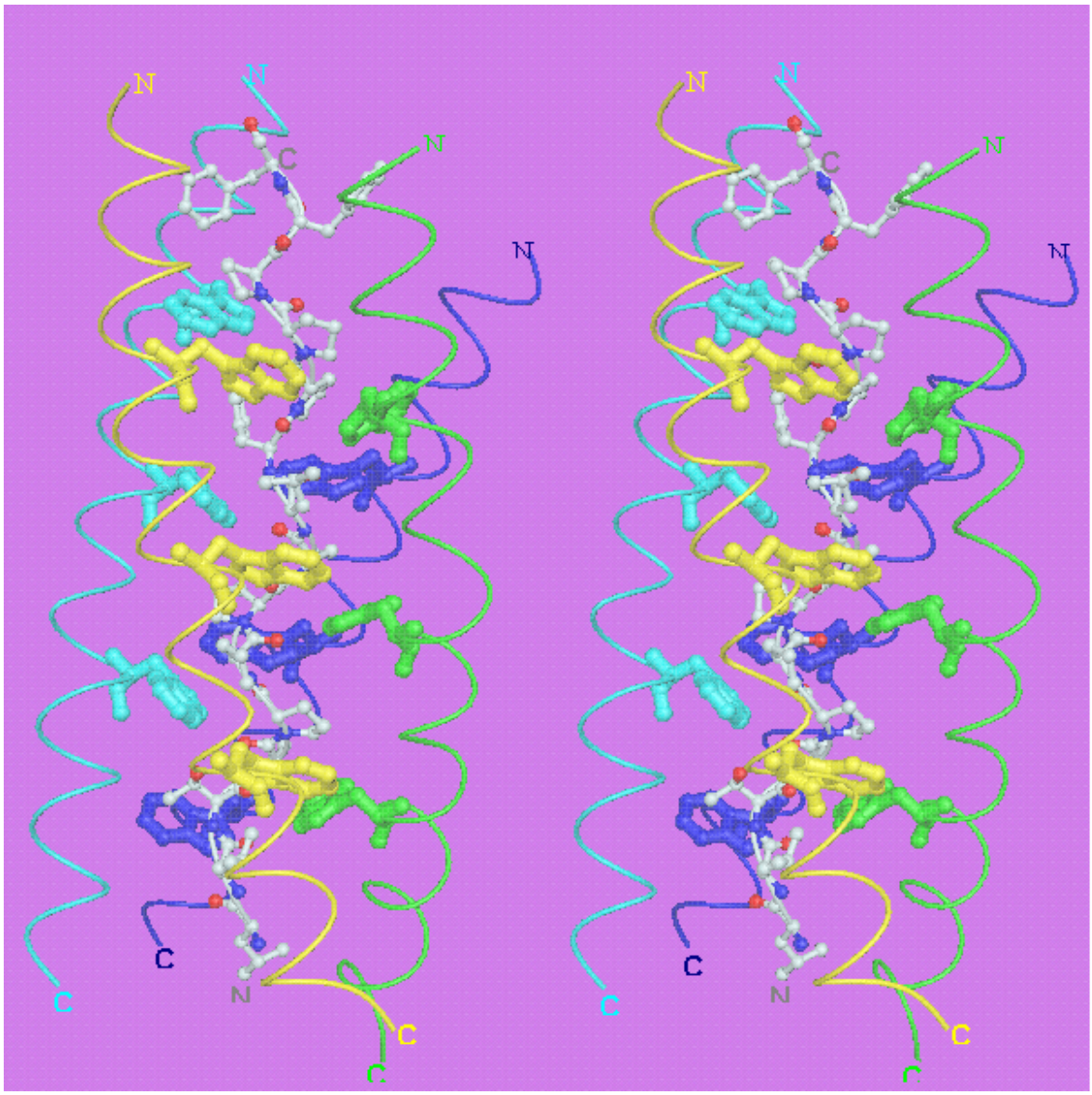

Figure 1. Stereo view of the crystal structure of the WAT/PRAD complex. Four parallel WAT peptides (shown as yellow, blue, green and cyan) associate with a single anti parallel PRAD peptide (shown in gray as a ball-and-stick model). Each WAT belongs to a different catalytic subunit of AChE, with PRAD being the N-terminal part of a collagen tail that organizes the enzyme in a tetrameric form. All four WAT chains contain an array of three highly conserved Trp residues (6 residues apart from each other), shown in ball-and-sticks format, each of which stacks against a Pro or a Phe residue in the single PRAD peptide. These interactions facilitate the formation of a super-helical structure, composed of four WAT helices twisted around the single PRAD peptide, which is in a polyproline II conformation.